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ORIGINAL PAPER

Study of DNA Light Switch Ru(II) Complexes : Synthesis, Characterization, Photocleavage and Antimicrobial Activity

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Abstract The three Ru(II) complexes of $[Ru(phen)_2dppca]^{2+}$ (1) $[Ru(bpy)_2dppca]^{2+}$ (2) and $[Ru(dmb)_2dppca]^{2+}$ (3) (where phen = 1,10 phenanthroline, bpy = 2,2-bipyridine, dmb = 2 ,2-dimethyl 2′,2′-bipyridine and polypyridyl ligand containing a single carboxylate functionality dppca ligand (dipyridophenazine-11-carboxylic acid) have been synthesized and characterized. These complexes have been shown to act as promising calf thymus DNA intercalators and a new class of DNA light switches, as evidenced by UV-visible and luminescence titrations with Co^{2+} and EDTA, steady-state emission quenching by $[Fe(CN)_6]^{4-}$ and KI, DNA competitive binding with ethidium bromide, viscosity measurements, and DNA melting experiments. The results suggest that 1, 2, and 3 complexes bind to CT-DNA through intercalation and follows the order 1>2>3. Under irradiation at 365 nm, the three complexes have also been found to promote the photocleavage of plasmid pBR322 DNA.

Keywords polypyridyl carboxylic group ligand · Ru(II) complexes · DNA-binding · Molecular light switch · Photocleavage

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Introduction

Polypyridyl Ru^{2+} complexes are useful non radioactive probes for structure elucidation of nucleic acids [1]. They have been found to be valuable as Photoluminescence properties with large molar absorption coefficients in the visible region [2, 3]. They have the potential to insert and stack between the DNA base pairs [4] and further develop into photochemical probes of DNA structure as well as anticancer drugs [5–7]. The interactions of CT-DNA with transition metal complexes, which contain planar polycyclic hetero aromatic ligands have been extensively studied [8–14]. A number of metal chelates are of current interest for important applications in nucleic acid chemistry as probes of DNA structure in solution, reagents for mediation of strand scission of duplex DNA under physiological conditions and chemotherapeutic agents and in genomic research [15–18]. Ruthenium complexes of various types are actively studied as metallodrugs as they are believed to have low toxicity and good selectivity for tumors; recently two Ru(II) complexes have also successfully completed phase-I clinical trials (ImH[transRu(III)Cl_4Im(DMSO)] [19, 20]. The binding of $[Ru(phen)_3]^{2+}$ remains an issue of rigorous debate [21] with factors such as size, shape and planarity of the intercalative ligand, and changing substituent group or substituent position on the intercalative ligand influencing the DNA-binding mechanism[22–25]. Since octahedral polypyridine Ru(II) complexes bind to DNA in three dimensions, the ancillary ligands also play an important role in the DNA-binding mechanism and behaviors [26–30]. Ru(II) polypyridyl complexes function as “molecular light switch” for DNA in aqueous solution, turning the switch on or off in the presence of DNA. As is well known, $[Ru(L)_2(dppz)]^{2+}$ (L = bpy (2,2-bipyridine) or phen = (1,10-phenanthroline); dppz = dipyrido[3,2-a:2,3-c]phenazine) is the most

extensively investigated complex as molecular "light switch" for DNA, because the complex shows negligible luminescence in aqueous solution at ambient temperature but displays strong photoluminescence in DNA solution [31]. Since many useful applications require that the complexes bind to DNA in an intercalative mode, much work has been done on modifying intercalative ligands, and the influence of ancillary ligands on DNA binding [32–35]. Herein, we report the synthesis and characterization of dppca ligand and their Ru(II) complexes $[Ru(phen)_2dppca]^{2+}$ (1) $[Ru(bpy)_2dppca]^{2+}$ (2) and $[Ru(dmb)_2dppca]^{2+}$ (3) (where bipy = 2,2′-bipyridine, dmb = 4,4′-dimethyl-2,2′-bipyridine, phen = 1,10-phenanthroline) (Fig. 1). The DNA-binding behaviors of complexes 1–3 are explored by absorption, emission spectroscopy viscosity and thermal denaturation studies and their abilities to induce cleavage of pBR-322 DNA. Antimicrobial studies are also for these complexes. There are many advantages in utilizing Ru(II) polypyridyl complexes in drug development, (1) the stable complexes with predictable structures can be prepared through reliable routes; (2) the shape selectivity of the complexes can be improved by functionalization of the ligands; (3) the knowledge of the biological effects of ruthenium complexes can be greatly developed. We focus our attention now on the design and synthesis of ruthenium (II) complexes with single carboxylate functionality of the polypyridyl ligand displaying strong DNA binding affinity and " molecular light switch" properties, so as to modify the properties of DNA. The results show that $[Ru(L)_2dppca]^{2+}$ possesses "molecular light switch" properties similar to those of $[Ru(bpy)_2(tpphz)]^{2+}$ [36, 37]. In this work, we have explored that the emission of $[Ru(L)_2dppca]^{2+}$ binding to DNA can be quenched by metal ions (Co^{2+}). Furthermore, the DNA-binding, DNA-photocleavage and spectral properties of these new Ru(II) complexes were carefully studied.

Experimental

Physical Measurements

UV-Visible spectra were recorded with an *Elico Biospectrophotometer, model BL198*. IR spectra were recorded in KBr discs on a *Perkin-Elmer FT-IR-1605 spectrometer*. ^{1}H and ^{13}C [^{1}H]-NMR spectra were measured on a *Bruker Z-Gradient single axis* fitted high resolution NMR Probe and 400 MHz Standard spectrometer using DMSO-d_6 as the solvent and TMS as an internal standard. Micro analysis (C, H and N) were carried out on a Perkin-Elmer 240 elemental analyzer. Fluorescence spectra were recorded with a *Elico spectrofluorimeter model SL 174.*

Materials and Methods

The compounds 1, 10, phenanthroline-5, 6-dione, $[Ru(bpy)_2Cl_2]$, $[Ru(dmb)_2Cl_2]$ and $[Ru(phen)_2Cl_2]$ were synthesized according to literature procedure[38, 39]. All chemicals were of reagent grade. A solution of calf thymus DNA in buffer gave a ratio of 1.8–1.9 UV absorbance at 260 and 280 nm indicating that DNA was sufficiently free of protein [40]. The DNA concentration per nucleotide was determined by using a molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [41].

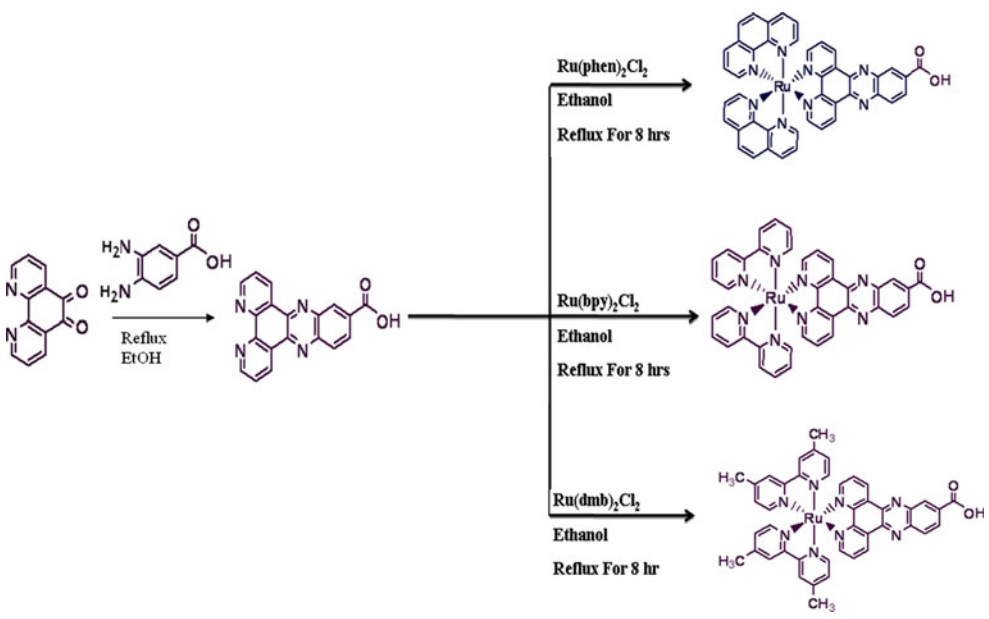

Fig. 1 Synthesis and structure of the complexes

Synthesis of Ligand

dppca (11-carboxy-dipyrido(3,2-a:2',3'-c)phenazine)

The ligand dppca was prepared by condensation reaction (0.5 g 2.38 mmol) of 1,10-phenanthroline-5,6-dione and (0.54 g 3.55 mmol) 3,4-diaminobenzoic acid were dissolved in 50 ml of ethanol and the mixture refluxed under nitrogen for 2 h. After cooling to room temperature a light brown, highly insoluble solid precipitated. Yield: (86%). Anal. Calc. for $C_{19}H_{10}N_4O_2$: C, 66.27; H, 2.93; N, 16.27. Found: C, 65.62; H, 2.60; N, 16.05. Es^+-MS Cal: 327, Found: 328.

[Ru(phen)$_2$ dppca] (ClO$_4$)$_2$ H$_2$O

$[Ru(phen)_2dppca]^{2+}$ was synthesized using a mixture of cis-$[Ru(phen)_2Cl_2]$ $2H_2O$ (0.11 g 0.25 mmol), dppca (0.0815 g 0.25 mmol) and ethanol 70 ml was refluxed under nitrogen for 8 h. It gave a clear red solution. Upon cooling a brown red precipitate was obtained by dropwise addition of a saturated aqueous $NaClO_4$ solution. After stirring the solution for 30 min, 5 ml ethyl ether was added and the solution left in the freezer for 24 h. A red solid precipitated, that was filtered off, washed with ethyl ether and dried. Yield: (73%). Anal. Calc. for $C_{43}H_{30}N_8O_{10}RuCl_2$: C, 47.06; H, 2.95; N, 11.10. C, 47.01; H, 2.70; N, 11.10. Fab^+mas: cal: 1004, found: 1003.

[Ru(bpy)$_2$ dppca] (ClO$_4$)$_2$ 0.5H$_2$O

This complex was obtained by a similar procedure to that described above, cis-$[Ru(phen)_2Cl_2]$ $2H_2O$ (0.13 g, 0.2 mmol) in place of cis-$[Ru(bpy)_2Cl_2]$ $2H_2O$. Yield: (88%). Anal. Calc. For $C_{39}H_{26}N_8O_{10}RuCl_2$: C, 45.49; H, 2.55; N, 10.88. Found: C, 45.79; H, 2.54; N, 10.84.

$\left[Ru(dmb)_2dppca\right](ClO_4)_2 2H_2O$

This complex was obtained by a similar procedure described above, cis-$[Ru(dmb)_2Cl_2]$ $2H_2O$ (0.116 g, 0.2 mmol) in place of cis-$[Ru(bpy)_2Cl_2]$ $2H_2O$. Yield: (82%). Anal. Calc. for $C_{43}H_{34}N_8O_{10}RuCl_2$: C, 47.56; H, 3.16; N, 10.32. Found: C, 46.01; H, 3.40; N, 11.10. Fab^+mas: cal: 1030, found: 1029. For all the complexes IR, ^{1}H- ^{13}C [^{1}H]-NMR, data are given in Tables 1 and 2.

DNA Binding Studies

Doubly distilled water was used to prepare tris buffer (5 mM Tris–HCl, 10 mM NaCl pH=7.1). The absorption titration of the complex in the buffer were performed by treating fixed concentration of complex (10 μM) with DNA. Complex-DNA solutions were allowed to incubate for 5 min before recording the absorption spectra. In order to evaluate the binding strength of the complex, the intrinsic binding constant K_b, with CT-DNA was obtained by monitoring the change in the absorbance at metal to ligand charge transfer (MLCT) band, with increasing concentration of DNA at 25°C. The intrinsic binding constant K_b, was calculated from Eq. 1 [42].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

Where [DNA] is the concentration of DNA, ε_a, ε_f and ε_b corresponds to the apparent absorption coefficient A_{obsd}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex in the fully bound form, respectively. In plots of [DNA]/ (ε_a-ε_f) versus [DNA]. K_b is given by the ratio of slope to the intercept.

In the emission studies fixed metal complex concentration (6 μM) was taken and to this varying concentration (0–150 μM) of DNA was added. The excitation wavelength was fixed and the emission range was adjusted before measurements. The fraction of the ligand bound was calculated from the relation $C_b = C_t[(F\text{-}F_0)/F_{max}\text{-}F_0)]$, where C_t is the

Table 1 ^{1}H NMR and IR data of complexes

Compound	IR-data (cm^{-1})				^{1}H-NMR (400 MHz, ppm DMSO-d$_6$, TMS)
	C = C	C = N	M-N (phen/bpy/dmp)	M-L	
dppca	1420	1575			9.1(s,1H), 8.8(d, 2H), 8.2(d,1H), 7.9(d , 1H), 7.7(d, 2H) 7.3 . (m,2H), 9.6 (COOH)
$[Ru(phen)_2dppca]^{2+}$	1451	1605	620	730	9.2(s,1H), 8.6(d, 6H), 8.2(d,1H), 7.9(d, 1H), 7.8(d, 6H) 7.61(d, 4H), 7.2(m, 6H), 9.7(COOH),
$[Ru(bpy)_2dppca]^{2+}$	1464	1602	625	766	9.5(s,1H), 9.3(s, 1H), 8.7(d,2H), 8.5(d, 4H), 8.3(d, 4H), 7.9(d, 4H), 7.6(m, 4H), 7.4(t, 2H), 7.1(t, 4H) 9.8(COOH)
$[Ru(dmb)_2dppca]^{2+}$	1451	1601	624	765	9.4(s, 1H), 8.9(d, 2H), 8.7(d, 4H), 8.4(d, 1H), 8.2(d, 4H), 7.9(d, 1H), 7.8(d, 2H), 7.6(t, 2H), 7.2(t, 4H) 10.0(COOH) & 2.2(4-methyl)

Table 2 ^{13}C [^{1}H] NMR data of ligand and complexes

Compound	^{13}C [^{1}H] NMR (100 MHz, ppm, DMSO-d_6, major peaks)
$[Ru(phen)_2dppca]^{2+}$	178.3, 172.6, 149.2, 145.0, 137.6, 132.3, 131.8, 126.3, 124.5
$[Ru(bpy)_2dppca]^{2+}$	172.5, 149.8, 145.5, 143.2, 141.2, 137.2, 131.1, 128.6, 121.6, 122.3
$[Ru(dmb)_2dppca]^{2+}$	174.5,159.8, 155.3, 154.6, 150.0, 147.3, 142.31, 138.5,133.3, 126.5, 121.0, 20.0(methyl)

total complex concentration, F is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is when the complex is fully bound to DNA. Binding constant (K_b) was obtained from a modified Scatchard equation [43], From a Scatchard plot of r/C_f vs r, where r is the C_b/[DNA] and C_f is the concentration of free complex.

Viscosity experiments were carried out on Ostwald viscometer, immersed in thermo stated water-bath maintained at 30±0.1°C. CT-DNA samples approximately 200 base pairs in average length were prepared by sonication in order to minimize the complexes arising from DNA flexibility [44]. Data were presented as $(\eta/\eta_0)^{1/3}$ versus concentration of [Ru(II)] / [DNA], where η is viscosity of DNA in the presence of the complex, and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t>100 s) corrected for the flow time of the buffer alone (t_0) [45]. Thermal denaturation studies were carried out with a *spectrophotometer*, by monitoring the absorbance at 260 nm taking the complex as (20 μM) and DNA as (120 μM) [46].

Salt dependence studies were performed in tris buffer by titrating preformed complex-DNA adduct with NaCl solution.

For the gel electrophoresis experiments, super coiled pBR-322 DNA (100 μM) was treated with Ru(II) complexes in tris buffer and the solution was then irradiated 2 h at room temperature with a UV lamp (365 nm,10 W). The samples were analyzed by electrophoresis for 2.5 h at 40 V on a 1% agarose gel in Tris- acetic acid-EDTA buffer, pH 8.2. The gel was stained with 1 μg /ml ethidium bromide and photographed under UV-light.

Microbial activity was performed by the standard disc diffusion method [47]. The complexes were screened for antibacterial activity against standard microorganisms such as E.coli, Pseudomonas aeruginosa, Staphylococcus aurous and antifungal activity against Neurospora crassa, Aspergillus niger, Aspergillus flavus. The Mueller Hinton agar was prepared and poured fresh into sterile Petri plates and allowed to dry, and inoculate 0.2 ml of bacterial culture which has 10^6 cells/ml concentrations. The complex was dissolved in DMSO to get a final concentration of 100 μl per disc. Each plate contains standard microorganisms with 3 different complexes (5 μl each compound) and standard antibiotics were also tested on these standard microorganisms as controls, and kept in the refrigerator for 5 min and these were transferred to the incubator at 37°C. After 24 h of incubation, the zone of inhibition of the complexes as well as standard antibiotics on standard microorganisms was checked. The minimum inhibitory concentrations for these complexes were measured. Dialysis experiment were conducted at room temperature with 3 cm^3 of CT-DNA (120 μM) sealed in a dialysis bag and 6 cm^3 of complexes (20 μM) outside the bag with the solution stirring for 24 h.

Results and Discussion

Fluorescence Spectroscopic Studies

The emission intensities of complexes from their MLCT excited state are found to depend on the DNA concentration. The emission spectra of the complexes 1–3 in the absence and in the presence of CT-DNA are shown in Fig. 2. In the absence of DNA and at 460 nm excitation, these three complexes emit relatively moderate luminescence in tris buffer at room temperature with the emission maxima at 598–600 nm. The change in emission may arise from the inter annular twisting between phen and other substituents. Here the introduction of dppca ligand may be responsible for the negligible luminescence. The luminescent properties of the complexes were perturbed when DNA was added to the complex solution, and binding of the complexes to DNA was found to increase the fluorescence intensity. Upon addition of CT-DNA to complex the fluorescence emission intensities of complexes 1, 2and 3 increased by a factor of

Table 3 Quenching data of Ru (II) complexes

Compound	$[Fe(CN)_6]^{4-}$ Complex: DNA			KI Complex: DNA		
	Complex alone	1:30	1:200	Complex alone	1:30	1:200
$[Ru(phen)_2dppca]^{2+}$	4361.3	608.8	39.2	143.8	25.48	4.69
$[Ru(bpy)_2dppca]^{2+}$	1254.6	679.5	45.6	107.9	32.1	7.68
$[Ru(dmb)_2dppca]^{2+}$	1089.5	725.9	47.8	85.4	42.5	11.26

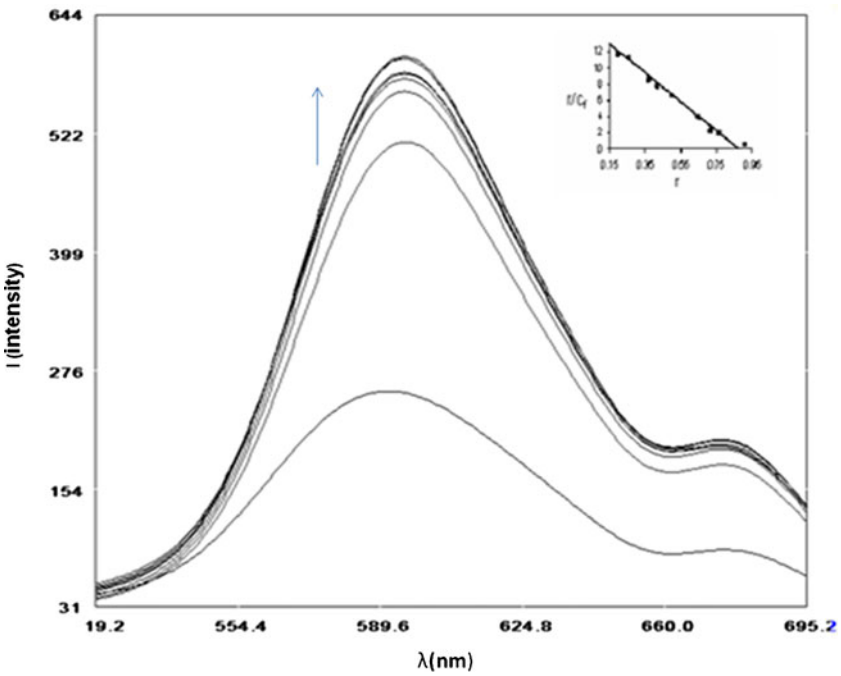

Fig. 2 Fluorescence emission spectra of complex $[Ru(phen)_2dppca]^{2+}$ (2 •M) in trisbuffer in the presence of CT– DNA

4.0, 3.8, and 3.4 times respectively. The intrinsic binding constant from fluorescence data was obtained from a modified Scatchard equation [43] through a plot of r/C_f vs r where r is the C_b/[DNA] and C_f is the concentration of the free metal complex. $C_b = C_t[(F-F_0)/F_{max}-F_0)]$, where C_t is the total complex concentration, F is the observed fluorescence emission intensity at a given DNA concentration, F_0 is the intensity in the absence of DNA, and F_{max} is the fully bound DNA to complex, binding constant is given by the slope. Scatchard plots for complexes have been constructed from luminescence spectra and binding constants (K_b) were 3.4×10^5, 2.1×10^5, and 1.2×10^5 M^{-1}

Quenching Studies

Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ and KI as quencher is also used to observe the binding of ruthenium(II) complexes with CT-DNA. Emission quenching with $[Fe(CN)_6]^{4-}$ in presence of DNA are shown in (Fig. 3) for $[Ru(bpy)_2dppca]^{2+}$ complex. The

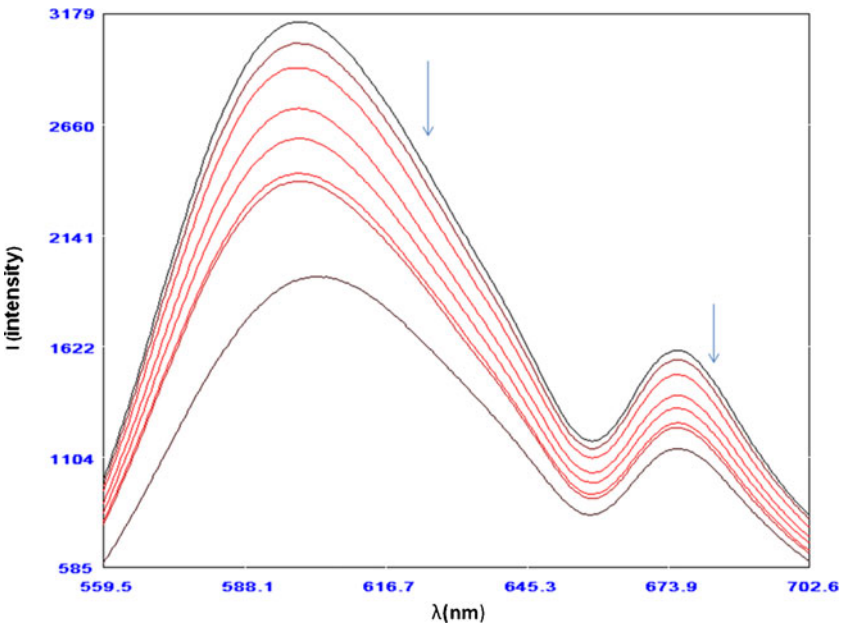

Fig. 3 Emission quenching of complex $[Ru(bpy)_2dppca]^{2+}$ in presence of DNA with $[Fe(CN)_6]^{4-}$

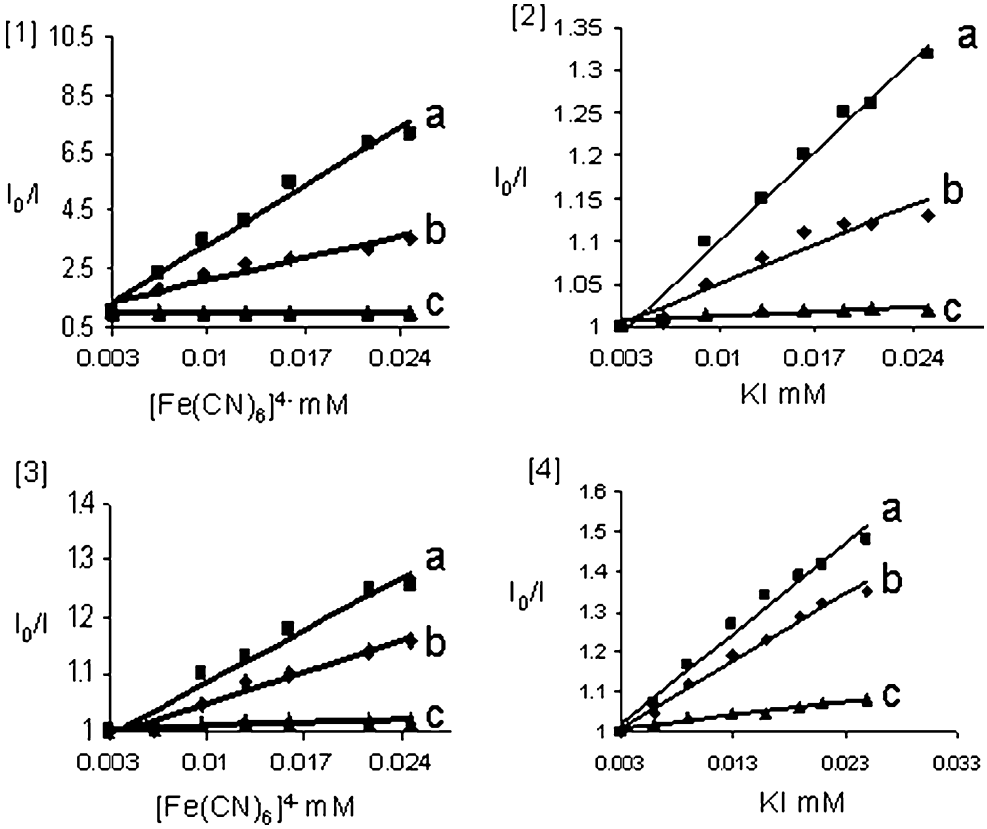

Fig. 4 Emission quenching of complexes $[Ru(phen)_2dppca]^{2+}$ (1, 2) and $[Ru(dmb)_2dppca]^{2+}$ (3, 4) with $[Fe(CN)_6]^{4-}$ and KI in the absence of DNA (**a**), presence of DNA 1:30 (**b**) and 1:200 (**c**) [Ru]=10 μM, $[Fe(CN)_6]^{4-}$= 0.1 M and KI =1 M

Stern–Volmer quenching constant (K_{sv}) can be determined by using Stern–Volmer equation [44]. $I_0 / I = 1 + K_{sv}[Q]$.

Where I_0 and I are the intensities of the fluorophore in the absence and presence of quencher respectively, Q is the concentration of the quencher, and K_{sv} is a linear Stern-Volmer quenching constant. Figure 4 shows the Stern–Volmer plots for the free complex in solution has high K_{sv} than in the presence of DNA. Highly negatively charged quencher is expected to be repelled by the negatively charged phosphate backbone, and therefore a DNA bound cationic complex should be less quenched by anionic quencher, than the unbound complex [48, 49]. All the complexes show linear Stern–Volmer plots. The K_{sv} value for the complexes in absence of DNA and in the presence of DNA (1:30 and 1:200) with $[Fe(CN)_6]^{4-}$ and KI are shown in (Table 4). More DNA binding shows less K_{sv} value. Comparing KI with $[Fe(CN)_6]^{4-}$, quenching is more with $[Fe(CN)_6]^{4-}$ because it is tetra negatively charged, where as KI is mono negative. From quenching studies it is clear that DNA binding ability of complexes follow the order 1>2>3 (Table 3).

Recovered luminescence of $[Ru(phen)_2dppca]^{2+}$ in the presence of Co^{2+} by EDTA

Interestingly, as shown in (Figs. 5 and 6), while adding EDTA into the buffer system containing $[Ru(phen)_2dppca]^{2+}$ with Co^{2+} ion, the emission intensity of the complex is recovered again. The phenomenon implies that the chelation of $[Ru(phen)_2dppca]^{2+}$ with Co^{2+} is weakened owing to the strong

Table 4 Antimicrobial activity of complexes (Zone of inhibition in mm)

Complex	Antibacterial (10 uM)			Antifungal		
	Zone of Inhibition			Zone of Inhibition		
	E.coli	P.aeruginosa	S.aureus	N.crassa	A.niger	A.flavus
$Ru(phen)_2dppca$	2.9	3.3	3.7	4.2	2.7	2.4
$Ru(bpy)_2dppca$	4.8	4.1	0.9	1.1	1.8	0.7
$Ru(dmb)_2dppca$	3.1	3.9	4.5	3.5	1	1.6

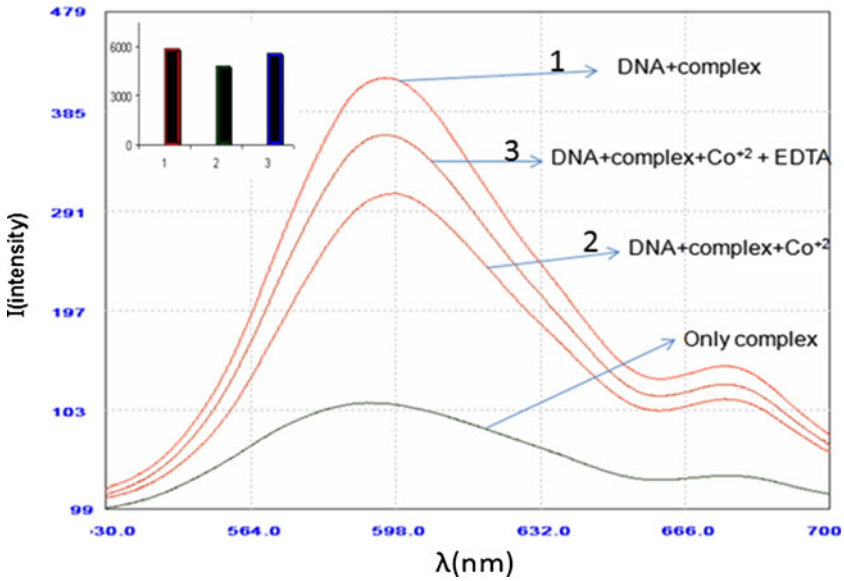

Fig. 5 DNA light switch on and off experiments showing the luminescence changes upon addition of Co^{2+}, EDTA to $[Ru(phen)_2\ dppca]^{2+}$ + DNA

coordination of Co^{2+} to EDTA, as a result, $[Ru(phen)_2dppca]^{2+}$ becomes free again [50] as shown in (Figs. 5 and 6). It is therefore interesting to investigate that the photoluminescence of DNA-bound $[Ru(phen)_2dppca]^{2+}$ could be tuned by successive introduction of Co^{2+} ions and EDTA. (Fig. 5) shows the decrease in the luminescence intensity of DNA-bound $[Ru(phen)_2dppca]^{2+}$ due to the interactions of Co^{2+} with DNA. While further adding EDTA into the buffer system containing DNA-bound $[Ru(phen)_2dppca]^{2+}$ with Co^{2+} ion, the emission intensity is recovered based on the strong coordination of Co^{2+} to EDTA. For example, the presence of 0.03 mM Co^{2+} could decrease the luminescence intensity by 11.5%, and the addition of the equimolar EDTA (0.03 mM) could result in the recovery of the luminescence up to 10.5.%. The value is more than 100%, possibly owing to the enhancement of EDTA on the luminescence of DNA-bound $[Ru(phen)_2dppca]^{2+}$ [51]. The luminescent change of DNA-bound $[Ru(phen)_2dppca]^{2+}$ in the presence of Co^{2+} and EDTA reveals the modulation of Co^{2+} and EDTA to luminescence intensities of DNA-bound $[Ru(phen)_2dppca]^{2+}$ (Fig. 6).

Electronic Absorption Titration

The binding of intercalative ligand to DNA has been characterized classically through absorption titration. Absorption

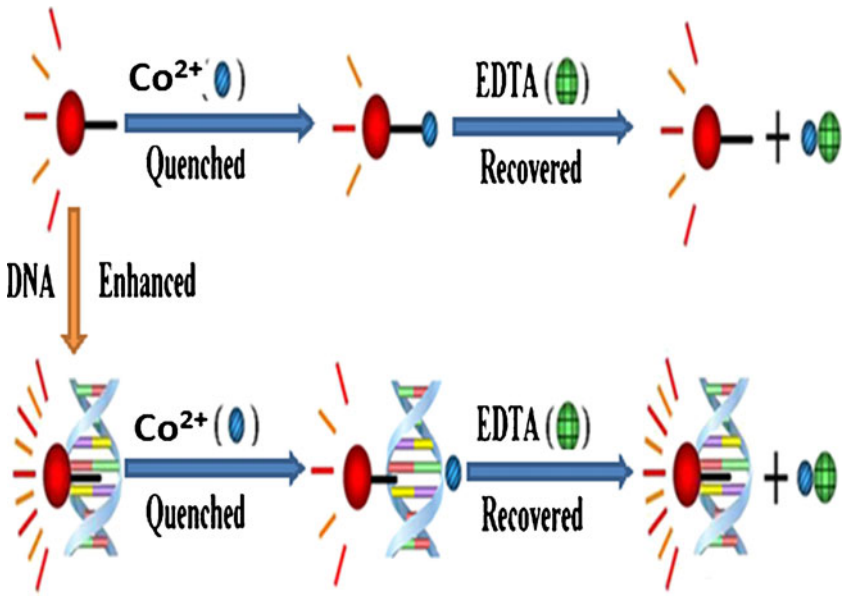

Fig. 6 Luminescence-modulation routes of $[Ru(phen)_2dppca]^{2+}$ in the absence and presence of DNA by Co^{2+} ion and EDTA, respectively

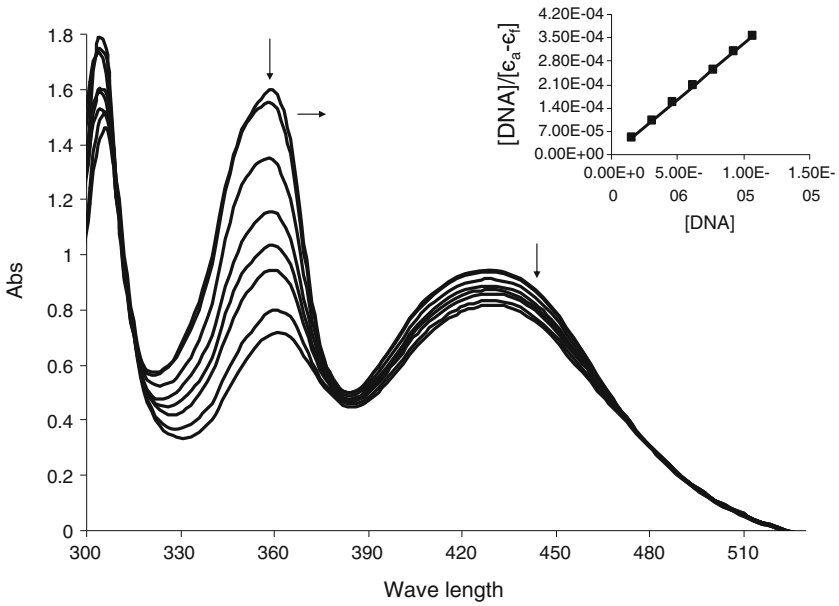

Fig. 7 Absorption spectra of Ru $(phen)_2dppca]^{2+}$ in tris-buffer upon addition of CT–DNA in absence (top) and presence of CT–DNA (lower) the [complex]=10–15 μm, [DNA]=0–100 μm. Insert plots of [DNA]/$(\epsilon_a-\epsilon_f)$ versus [DNA] for the titration of DNA with complex. Arrow shows change in absorption with increasing DNA concentration at 360 and 440 nm

titrations of Ru(II) complexes were done using a fixed ruthenium concentration to which increments of the DNA stock solution were added. The absorption spectra of complexes in the absence and presence of CT-DNA are given in (Fig. 7). The absorption spectra of complexes are characterized by distinct intense MLCT transitions in the vis-region, which are attributed to Ru (dπ) → bpy (π*), dmb (π*) or phen (π*) and Ru (d π) → dppca (π*) transitions. The bands between 290 and 350 nm for complexes 1–3 are attributed to intra-ligand (IL) π → π* transitions, and metal to ligand charge transfer (MLCT) in lower energy region around 450 nm. As the concentration increased, the MLCT bands of the complexes 1–3 exhibited hypochromism about 12.5%, 10.3%, and 7.8% as well as significant bathocromism about 10.0, 8.5 and 5.3 nm respectively. These results are similar to those reported earlier for various metallointercalators [52, 53]. Based on the observations we assume that there are some interactions between the complexes and the base pairs of DNA. To compare quantitatively the affinity of the complexes towards DNA, the intrinsic binding constants K_b of the Ru(II) complexes to CT-DNA were determined by monitoring the change of absorbance at λ_{max} of the complex in the visible region, with increasing concentration of DNA [54].

Intrinsic binding constants, K_b of complexes 1–3 are 3.4×10^5, 2.1×10^5, and 1.2×10^5 M^{-1}. These K_b values are

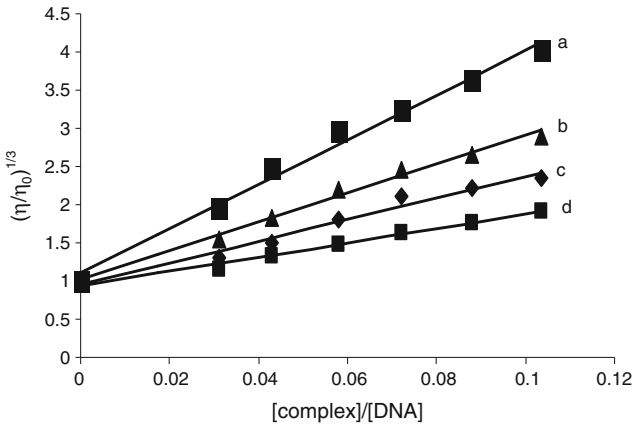

Fig. 8 Effect of increasing amounts of ethidium bromide (**a**), [Ru $(phen)_2dppca]^{2+}$ (**b**), $[Ru(bpy)_2dppca]^{2+}$ (**c**) and $[Ru(dmb)_2dppca]^{2+}$ (**d**) on the relative viscosity of calf thymus DNA at 30 ±0.1^0 C [DNA]=0.5 mM

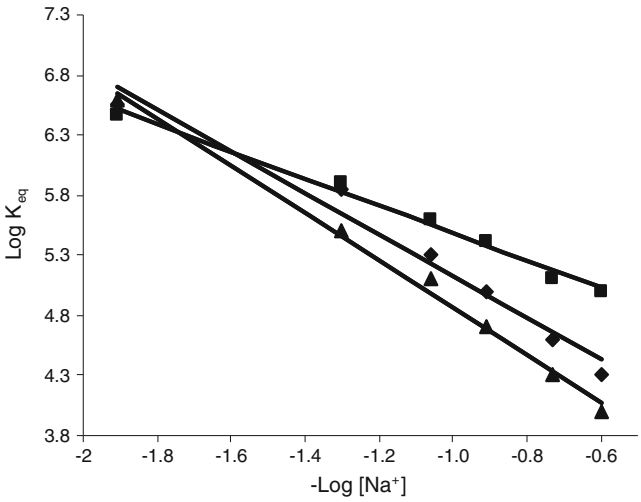

Fig. 9 Salt dependence of the equilibrium binding constants for DNA binding of complexes $[Ru(phen)_2dppca]^{2+}$ (▲), $[Ru(bpy)_2dppca]^{2+}$ (♦) and $[Ru(dmb)_2dppca]^{2+}$ (■). The line indicates the slope of the linear square fit to the data as −1.41, -1.20 and −1.13

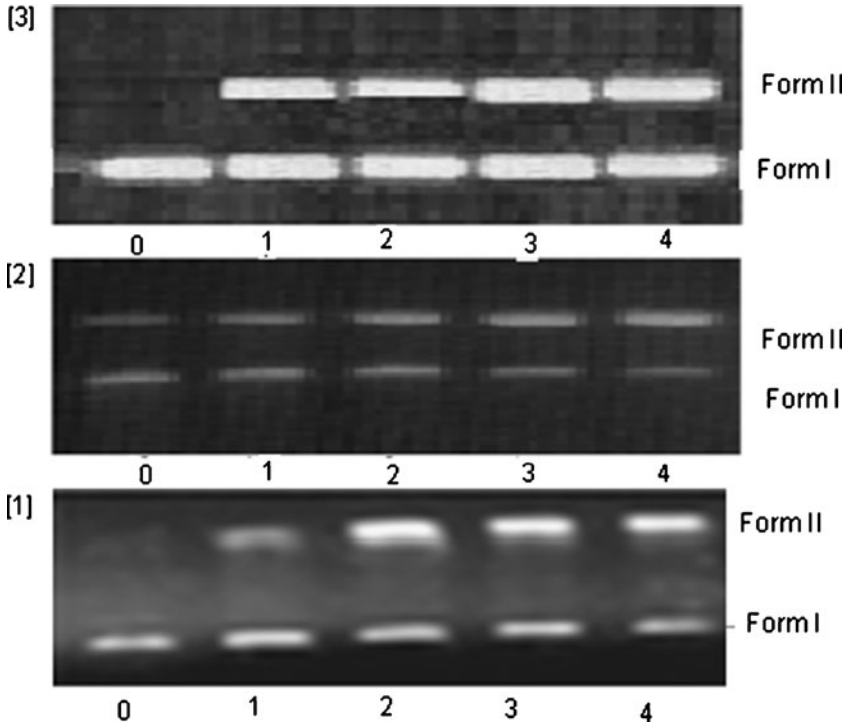

Fig. 10 Photocleavage studies of pBR322 DNA, in the absence and presence of complexes $[Ru(phen)_2dppca]^{2+}$ (1), $[Ru(bpy)_2dppca]^{2+}$ (2) and $[Ru(dmb)_2dppca]^{2+}$ (3) light after 30 min irradiation at 365 nm. Lane 0 control plasmid DNA (untreated pBR322), lane 1–6 addition of complexes in amounts of 10, 20, 30, 40, 50, 60 μL

smaller than those of classical intercalators, such as $[Ru(bpy)_2(dppz)]^{2+}$($K=1.6\times10^6$) [48] and $[Ru(bpy)_2(ppd)]^{2+}$, ($K=1.3\times10^6$) [55]. The difference between the binding constants of these complexes is due to different ancillary ligands. Electron deficient rings interact more strongly with polyanion (DNA) than electron rich rings. Complex 3 show the less binding strength to double-helical DNA. Due to the presence of methyl groups on the 4 and 4′ positions of the ancillary ligand, dmb causes steric hindrance when the complex intercalates into the DNA base pairs and methyl groups donates electrons to Ru^{2+} and make electron denser, hence decreasing the binding constant and follows the order 1>2>3. The different K_b values obtained by the two titration methods of measurements (absorption and fluorescence titration) are in good agreement with that of absorption spectroscopy.

Viscosity Studies

The DNA binding modes of complexes were further investigated by viscosity measurement. The viscosity measurements of DNA is regarded as the least ambiguous and the critical test of a DNA binding model in solution and provides strong evidence for intercalative DNA binding mode [44, 56]. A classical intercalation model results in lengthening the DNA helix as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial non classical intercalation of ligand could bend (or kink) the DNA helix, and reduce its effective length [45]. For example, under appropriate conditions, intercalation of dye like EtBr causes a significant increase in the overall DNA length. The effects of the complexes on the viscosity of rod-like DNA comparing with EtBr are shown in (Fig. 8). Though the intercalating ligand is same in all complexes, there is small difference in the viscosity, this is due to the difference in the ancillary ligands. These further suggest that three Ru(II) complexes show an intercalative binding mode to CT-DNA, which parallel the absorption titration results. The increased degree of viscosity, also supports the order of binding of the complexes to DNA as determined by other methods which follows the order EB >1>2>3 (Fig. 8).

Table 5 Minimum inhibition concentration (MIC) of complexes (μg/ml)

(IC-50: 50% growth inhibition concentration)

Complex	Minimum Bactericidal conc (ug/mL)					IC-50
	E.coli	P.aeruginosa	S.aureus	N.crassa	A.niger	A.flavus
$Ru(phen)_2dppca$	7.8	6.2	6.2	1.4 mM	–	–
$Ru(bpy)_2dppca$	3.1	3.1	0.78	–	–	–
$Ru(dmb)_2dppca$	6.2	1.56	0.31	2.6 mM	–	–

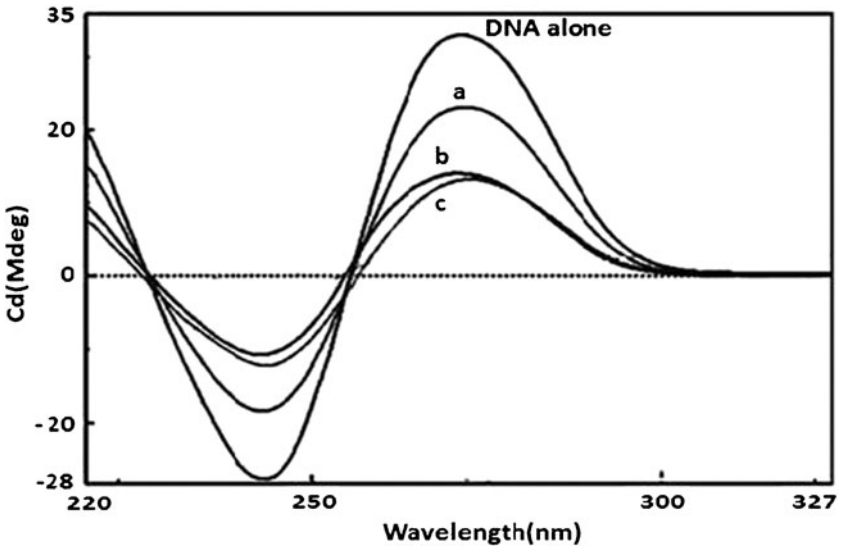

Fig. 11 CD spectra of CT-DNA in the absence (DNA alone) and presence of complexes $[Ru(phen)_2dppc]^{2+}$(a), $[Ru(bpy)_2dppca]^{2+}$ (b) and $(Ru(dmb)_2dppca]^{2+}$(c)

DNA Melting Studies

As Intercalation of the complexes into DNA base pairs causes stabilization of base stacking and hence raises the melting temperature of the double standard DNA. The DNA melting experiments are useful in establishing the extent of intercalation. All the three complexes were incubated with CT-DNA, and raised the temperature 25–80°C and the OD at 260 nm was monitored [57]. Here T_m of CT–DNA was found to be 60.2°C in tris-buffer, with addition of the complex (30 μL) to DNA, T_m increases to 68.2±0.1°C, 70.8±0.1°C and 72.3±0.1°C. Binding of complexes to DNA lead to an increase in T_m of DNA, in the order 3<2<1.

Salt Dependence Studies

The salt dependence binding of complexes 1, 2 and 3, to DNA is shown in Fig. 10. As the concentration of salt (NaCl) increases the binding constant decreases. The dependence of binding constant for these complexes upon Na^+ concentration is a consequence of the linkage of complex and Na^+ binding to DNA and may be analyzed by polyelectrolyte theory [58]. From the theory, the slope of the lines in (Fig. 9) provides an estimate of Zψ, where ψ is the fraction of counter ions associated with each DNA phosphate(ψ = 0.88 for DNA) and Z is the charge on the complex (Z = +2). The slopes of the lines in Fig. 9 are being −1.41, -1.20, and −1.13 for 1, 2 and 3 complexes respectively. These values are less than the theoretically expected values of Zψ (2×0.88=1.76). Such lower values could arise from coupled anion release or from change in complex or DNA hydration upon binding [45]. The knowledge of Zψ allows for a quantitative estimation of the non electrostatic contribution to the DNA binding constant for these complexes.

Anti-Microbial Activity

The antimicrobial screening data (Table 4) show that the complexes possess good antibacterial and antifungal properties. Metal complexes showed enhanced antibacterial and fungal activity. Chelating tends to make the chelating ligands more potent bactereostatic agent, thus inhibiting the growth of bacteria and fungi upon complexation the lipophilic character increased and favors the permeation through the layer of the bacterial membranes. The change in the antibacterial strength of complexes towards different bacteria depends either on the impermeability of the cells of the microorganism or the difference in ribosome of microbial cells. Concentration is also an important factor in increasing the antimicrobial activities. Antimicrobial activities increase with increase in the concentration of the complexes, and the IC-50 values derived from the experimental data were summarized in (Table 5).

Photo-Activated Cleavage of pBR322 DNA by Ru(II) Complexes

A number of metal polypyridyl complexes have been studied in relation to their DNA photocleavage behaviors [59, 60] the cleavage reaction on plasmid DNA can be monitored

Table 6 CD data for the interaction of CT DNA with complex 1,2 & 3

S.NO	Complexes	Positive peak	Negative peak
1	DNA alone	271 nm	−246 nm
2	1	272 nm	−242 nm
3	2	273 nm	−244 nm
4	3	273 nm	−245 nm

by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration is observed for the supercoiled form (Form-I). If scission occurs on one strand (nicking), the supercoil relax to generate a slower-moving open circular form (Form-II) [61]. If both strands are cleaved, a linear form (Form-III) is generated that migrates between forms I and II, as seen in (Fig. 10). Control photoreactions with DNA alone (lane 0) results in little or no DNA cleavage. In contrast, photoreactions at 365 nm results in significant production of nicked DNA depending on the concentration of the complexes used. It can be seen that by increasing the concentrations of the complex, Form-II increases gradually, while Form-I diminishes.

Equilibrium Dialysis Experiments

A difference in biological activities between the enantiomers of an optically active metal complex has been noted in many examples such as toxicity and drug efficiency [62, 63], in order to understand such effects better at the level of molecular interaction. It is therefore important to examine enantiomeric effects on the DNA binding of a metal complex. According to the insertion model proposed by Barton and coworkers [45, 64], the Δ enantiomer of the complex, a right handed propeller-like structure, will display a greater affinity than the Λ enantiomer with the right-handed CT-DNA helix, due to the appropriate steric matching. This discrimination can be observed via equilibrium dialysis experiments and provide strong evidence in support of intercalation. The CD spectrum of CT DNA exhibits a positive band at 271 nm due to base stacking and a negative band at 242 nm due to the helicity of DNA. In the presence of the complex an increase in the molar eliplicity values of the both positive and negative band of the CT DNA is observed (Fig. 11). Those significant changes indicate conformational changes and unwinding of DNA base pairs with destabilization of the DNA double helix, which is consistent with DNA intercalation mode suggested above (Table 6).

Conclusion

In summary, Ru(II) complexes $[Ru(phen)_2dppca]^{2+}$ (1) $[Ru(bpy)_2\ dppca]^{2+}$ (2) $[Ru(dmb)_2\ dppca]^{2+}$ (3) have been synthesized and characterized by elemental analysis, IR, mass, ^1H and ^{13} C-NMR spectra . Spectroscopic studies and viscosity experiments supported that the complexes can intercalate into DNA base pairs via dppca ligand. The electrophoresis experiment showed that the interaction of the complexes with DNA induced strand breakages, when irradiated at 365 nm the three Ru(II) complexes are efficient photocleavers of the plasmid pBR322 DNA. Complex 1 and 2 are found to show activity slightly more than the standard drugs against bacterial species. The reported complexes exhibit good antimicrobial activity. In addition, in the presence of Co^{2+}, the emission of DNA-$[Ru(L)_2\ dppca]^{2+}$ can be quenched. The experimental results show that $[Ru(L)_2dppca]^{2+}$ exhibited the DNA "light switch" properties. The present results should be of value in further developing luminescence DNA probe.

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